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Compatible and Incompatible *Xanthomonas* Infections Differentially Affect Herbivore-Induced Volatile Emission by Pepper Plants

Yasmin J. Cardoza · James H. Tumlinson

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Abstract Recent studies have alerted us to the potential for conflicts between pathogen- and herbivore-induced plant defenses. The lack of studies on the induced chemical changes that simultaneous insect and pathogen attacks have on the host plant has become apparent. In the present study, we found that pepper plant volatile profiles can be differentially induced by compatible and incompatible bacterial infection and beet armyworm (BAW) damage when applied alone or in combination upon the same host. We also found that plants under simultaneous compatible bacterial and BAW attack are able to produce volatiles in quantities greater than those produced by healthy plants in response to BAW feeding. In contrast, plants exposed to the incompatible pathogen challenge showed a total volatile release below the level of healthy plants exposed to BAW damage. This suppression of BAW-induced volatiles coincided with increased methyl salicylate production from incompatible bacteria-infected plants. Feeding choice experiments revealed that, when given a choice, BAW larvae fed significantly more on leaves of plants infected with the incompatible bacteria as soon as 2 d after inoculation, while a significant increase in insect feeding on the plants infected with the compatible bacterial strain was not seen until day 4 after inoculation. Additionally, survival for third instars to pupation was significantly higher when feeding on infected plants than on

Y. J. Cardoza (✉)
Department of Entomology and Nematology, University of Florida,
Gainesville, FL 32611-0620, USA
email: cardoza@entomology.wisc.edu

J. H. Tumlinson
Center for Medical Agricultural and Veterinary Entomology, United States Department of Agriculture,
Agricultural Research Service, 1700 SW 23rd Drive, Gainesville, FL 32608, USA
email: jht2@psu.edu

Y. J. Cardoza
345 Russell Laboratories, 1630 Linden Drive, Madison, WI 53706, USA

J. H. Tumlinson
Department of Entomology, 111 Chemical Ecology Laboratory, The Pennsylvania State University,
University Park, PA 16802, USA

healthy plants, regardless of compatibility. These results are indicative of lowered herbivore defenses due to disease progression on the plants.

Keywords Pepper · Volatiles · Methyl salicylate · Insect feeding · Beet armyworm · Bacterial infection · Induced defense

Introduction

Plants have innate defense mechanisms that can be differentially activated in response to insect and pathogen attack. For example, plants may contain significant amounts of constitutive secondary metabolites including phenolics, terpenoids, and steroids, which are toxic to invading organisms. In addition to constitutive chemical defenses, there are also compounds that are actively produced or induced after penetration of the tissues has occurred. These inducible defenses are energetically expensive and are only produced upon specific recognition of an invading organism (Ryan and Jagendorf, 1995; Hahn, 1996; Scröder, 1998). Plants that are able to recognize an invading organism activate responses including a rapid localized cell death, also known as a hypersensitive response (HR), at the site of penetration and activation of biochemical defense responses. Biochemical responses include the following: production of reactive oxygen species, structural changes in the cell wall, accumulation of defense-related proteins, and phytoalexin biosynthesis. These chemical defenses can directly affect the development and survival of attacking organisms (Mür et al., 1997). Upon attack, plants may also produce secondary defense chemicals, volatile organic compounds (VOC's), which are released into their immediate airspace. These VOC's are produced by plants in response to attack by herbivores (McCall et al., 1994; Loughrin et al., 1995; Röse et al., 1996; Paré and Tumlinson, 1997) and pathogens (Cardoza et al., 2002; Huang et al., 2003). These VOC's are of great ecological relevance because they have been shown to attract parasitoids of insect pests (Turlings et al., 1991, 1993; Röse et al., 1998; Cardoza et al., 2003a) and to hinder pathogen development (Zeringue and McCormick, 1989, 1990; Zeringue et al., 1996; Cardoza et al., 2002).

While the responses of plants to individual attacks by insects and pathogens have been well studied, regulation of defense mechanisms that help plants cope with multiple stress factors remains to be elucidated. Similarly, it is not yet clear what defense pathways are involved in the production of VOC's by plants in response to combined insect and pathogen attack. Recent studies suggest that activation of plant defenses by pathogens interferes with plant defenses against herbivorous arthropods and *vice versa* (Karban et al., 1987; Bostock, 1999; Felton et al., 1999; Fidantsef et al., 1999; Stout et al., 1999). In light of these possible conflicts, studies on the interactions between insect and pathogen species, and the induced chemical changes that such interactions have on the host plant are needed.

An understanding of the dynamics involved in triggering plant innate defense mechanisms can lead to the development of crop protection based on genetic or chemical manipulation of such responses. The key to exploitation may lie in our ability to decipher the extent to which the biochemical defense cascades triggered within the plant by pathogen and herbivore damage interfere with one another, and to minimize these trade-offs. In this study, we evaluated VOC emissions elicited in bell pepper plants, *Capsicum annuum* L., by compatible and incompatible strains of the leaf spot pathogen, *Xanthomonas campestris* pv. *vesicatoria*, pepper race 1 (XCVP3; compatible) and tomato race 1 (XCVT1; incompatible). We also evaluated the effect of infection by these pathogens on the plants' ability to produce VOC's in response to feeding by beet armyworms (BAW), *Spodoptera exigua* Hübner. VOC

profiles of healthy plants and plants exposed to attack by the insect and the pathogen, individually and in combination, were analyzed and compared. The effect of infection with the two types of bacteria on the insects' feeding preference and larval development, from third instar to pupa, was also evaluated.

Methods and Materials

Plant and Insect Material

Pepper seeds "Early CalWonder" (Grimes, Concord, OH, USA) were sown in pairs in 1-gal pots (16 cm diam) containing Metromix 300 (Scotts-Sierra Horticultural Company, Marysville, OH, USA). Plants were grown in an insect-free greenhouse with natural light, under Florida summer conditions (14:10 light/dark cycle). The greenhouse temperature was kept between 25°C and 30°C. After emergence, seedlings were thinned to 1 individual per pot. Each plant received 100 ml liquid fertilizer [20:20:20 (N/P/K); Peters, W.R. Grace, Fogelsville, PA, USA] every 2 wk starting on the first week after emergence. Six-wk-old pepper plants with eight fully developed leaves were used in all experiments. BAW eggs were obtained from the rearing facilities at the USDA-IBPMRL (Tifton, GA, USA). Larvae were reared on a pinto bean-based artificial diet following the methodology described by King and Leppla (1984). Insects were maintained on a 14:10 L/D cycle and maintained at 28°C. Third instars were used in all experiments.

Bacterial Culture and Plant Inoculation

The initial cultures of *X. campestris vesicatoria*, pepper race 3 (XCVP3; compatible) and tomato race 1 (XCVT1; incompatible), were obtained from Jeffrey B. Jones (Department of Plant Pathology, University of Florida, Gainesville, FL, USA) and were grown on nutrient agar (NA) Petri plates. Subsequent cultures were started by using nutrient broth, and were stored in 15% glycerol at −70°C for later use. Viable cells for plant inoculation were obtained incubating 100 µl of the frozen culture in 50-ml conical centrifuge tubes containing 15 ml nutrient broth. Tubes were placed into a 200-rpm orbital shaker within a biological incubator kept at 28°C for 18 hr. Cells were harvested by spinning at 3000 × *g* for 10 min, and pellets were resuspended in tap water. Bacterial cell concentration was estimated by measuring absorbance with a spectrophotometer set at 600 nm. Concentration was adjusted to 10^7 CFU/ml with water and 400 µl/l of Silwett L-77 were added to help cell penetration into the leaf. Plants were inoculated by dipping their aerial portions into the bacterial suspension for 20 sec. Control plants were mock-inoculated by dipping in the water Silwett L-77 mixture without bacterial cells.

Volatile Collections from Pathogen Infected and BAW-Damaged Peppers

Plant treatments consisted of (1) control (uninfected/undamaged) dipped in Silwett L-77 water, as described above; (2) BAW-damaged; (3) XCVP3-infected; (4) XCVT1-infected; (5) XCVP3-infected plus BAW damage; and (6) XCVT1-infected plus BAW damage. Plants were inoculated with bacteria as described above 12 hr before the start of the first sampling period. BAW-damaged plants were also exposed to feeding by six third instars within the volatile collection chambers 12 hr before the start of the first sampling period.

The aerial portion of each intact, nonexcised plant was contained within a cylindrical glass chamber with a guillotine-type Teflon base, which surrounded the plant stems (Röse et al., 1996). Purified air was pushed into the top of the chamber at a rate of 5 l min^{-1}. Samples of air from the whole-plant chambers were collected by pulling air at 1 l min^{-1} through 25 mg Super Q (80–100 mesh) (Alltech, Deerfield, IL, USA) adsorbent traps located around the bottom of each chamber (12). Volatiles were sampled for 4 d in three consecutive periods each day: (1) 6:00 A.M.–12:00 P.M., (2) 12:00–6:00 P.M., and (3) 6:00 P.M.–6:00 A.M. The experiment was set up in single replicates and repeated on different days for a total of six replicates. Data presented as total volatile production are for combined diurnal collection periods (1 + 2). Total volatile production represents the sum of the amounts of all the individual compounds produced by a given treatment.

Sample Extraction and Analysis

Compounds from individual traps in the volatile collection experiments were eluted with 170 μl dichloromethane (GC/GC-MS Solvent; B&J, AlliedSignal, Inc, MI, USA), and then 400 ng each of *n*-octane and nonyl acetate were added to each eluted sample as internal standards. Samples were analyzed by gas chromatography with flame ionization detection (HP5890 Gas Chromatograph, HP7673 auto sampler; Hewlett-Packard, Palo Alto, CA, USA) equipped with a 15 m (H) × 0.25 mm (ID) × 0.25 μm film thickness DB-1 capillary column (Quadrex, New Haven, CT). The splitless mode injector system was set at 220°C, the column oven was maintained at 40°C for 1 min after injection, and then programmed at 14°C min^{-1} to 180°C. The carrier gas used was helium at an average flow velocity of 19 cm sec^{-1}.

For identification of volatile compounds, selected samples were analyzed via GC/MS (HP 6890/5893) in electron impact mode. Individual compounds were identified by comparing their retention times and mass spectra to those of commercially obtained authentic samples and/or by comparing their mass spectra to those available in a database from the Environmental Protection Agency/National Institute of Standards and Technology.

Effect of Bacterial Infection on BAW Larval Feeding and Performance

To determine if herbivore-induced defenses were compromised in bacterially infected plants, we conducted BAW feeding choice experiments at different times during disease development on the plants. Additionally, the performance of BAW, from third instar to pupa, on healthy and bacteria-infected peppers was evaluated.

To determine the feeding preference of BAW larvae for plants at different times during infection with both bacterial strains, insects were given a choice between leaves from healthy plants and leaves of plants 0, 2, 4, or 6 d after bacterial inoculation. Plants with eight fully developed leaves were used. The fifth oldest leaf of an infected plant was paired with its counterpart from a healthy plant by confining them, while still attached to the plants, side by side within Petri dish clip cages with six third instar BAW larvae (Alborn et al., 1996). Larvae were allowed to feed on the leaves for 24 hr. Leaves exposed to the feeding were photocopied, and the leaf images were scanned and imported into an imaging software program (ImagePC beta version 1; Scion Corporation, Frederick, MD, USA) to calculate leaf area eaten and leaf area remaining for each of the treatments. This experiment was repeated over time for a total of six replicates.

To evaluate larval performance on healthy and infected pepper, plants were individually placed within 46 × 46 × 46 cm plexiglass cages into which 6 third instar BAW were introduced. Although insects were not weighed at the time of introduction, care was taken so that

all larvae used were newly molted third instars. Infected plants had been inoculated 3 d before the beginning of the experiment. Cages were kept in the greenhouse throughout the experiment. One plant provided more than enough food for all insects to develop until pupation. Insects were observed daily and were removed from the plants when they reached the wandering stage and no more feeding activity was observed. All insects from each of the treatments were collectively placed into a Petri plate labeled to indicate plant treatment and replicate number, and kept in the incubator until pupation. At the time of pupation, the number of surviving pupae and their weights were recorded. Eight replicates of this experiment were set up at one time in the greenhouse. This experiment was set up at two different times, by using either the compatible or the incompatible strain of the pathogen to inoculate plants.

Statistical Analyses

Data for total volatile emission were analyzed with ANOVA (SAS Institute, 1996). Significant ANOVAs were followed by Tukey's mean separation test. Data for BAW feeding preference were analyzed by paired *t* test (SAS Institute, 1996) for each day. Data for performance of BAW on healthy and bacteria-infected pepper were subjected to an analysis of variance (ANOVA; SAS Institute, 1996).

Results

Effect of BAW Feeding and Compatible and Incompatible Bacterial Inoculations on Volatile Production

Hypersensitive response symptoms were observed on the XCVT1-infected plants as early as 24 hr after inoculation, whereas chlorotic spots on the leaves in response to XCVP3 were seen only 3-4 d after inoculation. Volatile emission was highest during the second collection period (12:00–6:00 P.M.) and lowest during the third collection period (6:00 P.M.–6:00 A.M.) for the length of the experiment, regardless of plant treatment. Because of the high degree of variability, VOC emissions from the two diurnal collection periods were not different and were combined. The volatile blend emitted by plants under different biotic challenges consisted of mono- and sesquiterpenes, lipoxygenase products, and methyl salicylate. However, there were differences in the amounts of each of these compound classes among the different treatments. For instance, total monoterpene release by plants damaged by BAW alone was the highest, followed by plants damaged by combinations of the herbivore and compatible and incompatible bacteria, respectively (Table 1). Monoterpene release by plants infected with the compatible bacterial strain were comparable to those of control plants; however, plants infected with the incompatible bacteria released higher amounts of these compounds on days 3 and 4 compared to controls (Table 1). Sesquiterpene release was significantly higher for plants under the XCVT1/BAW treatment during all 4 d sampled (Table 1). Plants exposed to XCVT1 and XCVT1/BAW had their highest sesquiterpene release on the first day of sampling, decreased on day 2, and remained constant during days 3 and 4 (Table 1). Release of sesquiterpene compounds by BAW damaged plants was equivalent to that released by plants under XCVP3/BAW attack, but higher than those of XCVP3-infected and control plants. Emission of sesquiterpenes by plants under XCVP3 infection alone was equivalent to that of the controls. Production of LOX products was most prevalent in the emissions of plants exposed to BAW damage alone or in combination with either bacterium (Table 1). Methyl salicylate was released by plants exposed to infection by either bacterium alone or

Table 1 Biosynthetic classes of volatiles emitted over a four day period by pepper plants subjected to different BAW/*Xanthomonas* treatments

Treatment	Volatile release (ng plant^{-1} h^{-1})[a]			
	MonoT[b]	LOX[b]	SesqT[b]	Methsal[b]
Day 1				
Control	242 ± 15.8a	2 ± 2.0a	13 ± 6.2a	0 ± 0.0a
XCVP3	299 ± 26.1a	3 ± 2.1a	39 ± 16.4a	16 ± 10.5ab
XCVT1	568 ± 53.4b	8 ± 7.7a	479 ± 262.0c	67 ± 44.6bc
BAW	1739 ± 38.6c	38 ± 8.6b	78 ± 21.6b	0 ± 0.0a
XCVP3BAW	2409 ± 81.5c	42 ± 13.8b	73 ± 17.6b	21 ± 96.5ab
XCVT1BAW	894 ± 45.2b	51 ± 22.4b	948 ± 318.9c	184 ± 73.4c
Day 2				
Control	249 ± 13.3a	2 ± 1.8a	8 ± 3.9a	0 ± 0.0a
XCVP3	271 ± 20.4a	9 ± 8.8a	19 ± 6.9a	5 ± 2.3a
XCVT1	429 ± 31.1b	6 ± 3.4a	75 ± 39.0b	111 ± 41.5b
BAW	5873 ± 185.9d	142 ± 91.6b	84 ± 22.5b	0 ± 0.0a
XCVP3BAW	10,373 ± 394.0e	151 ± 65.7b	147 ± 52.1bc	38 ± 3.8b
XCVT1BAW	1390 ± 44.1c	39 ± 17.5ab	308 ± 83.9c	71 ± 38.8c
Day 3				
Control	325 ± 24.7a	7 ± 6.8a	9 ± 4.3a	0 ± 0.0a
XCVP3	270 ± 15.8a	4 ± 2.0a	20 ± 7.8a	154 ± 21.6bc
XCVT1	787 ± 73.3b	15 ± 14.5a	46 ± 16.4a	113 ± 59.6b
BAW	4718 ± 203.4c	178 ± 91.6b	130 ± 62.6b	0 ± 0.0a
XCVP3BAW	8970 ± 249.0e	253 ± 96.1b	143 ± 38.9b	33 ± 12.2b
XCVT1BAW	3571 ± 147.4d	167 ± 69.5b	554 ± 156.8c	510 ± 112.5c
Day 4				
Control	133 ± 9.4a	7 ± 6.3a	9 ± 3.6a	0 ± 0.0a
XCVP3	319 ± 28.4b	2 ± 1.5a	14 ± 4.8a	198 ± 16.7b
XCVT1	1340 ± 78.5c	27 ± 14.8b	50 ± 29.1a	62 ± 43.2ab
BAW	7983 ± 345.3d	368 ± 191.9c	150 ± 53.4b	0 ± 0.0a
XCVP3BAW	10,533 ± 409.6d	317 ± 198.6c	191 ± 81.2b	2936 ± 1458.9d
XCVT1BAW	3601 ± 181.8c	171 ± 118.5c	678 ± 317.2c	472 ± 109.1c

MonoT: Monoterpenes; SesqT: sesquiterpenes: LOX: lypoxygenase products; Methsal: methyl salicylate.

[a] Mean ± SE for emissions during period 2 (12:00–6:00 P.M.).

[b] Values within days followed by different letters denote significant differences in emission of compound classes between treatments.

in combination with herbivore damage (Table 1). However, plants under the incompatible bacterial treatment started releasing this compound on day 1 (XCVT1/BAW) or day 2 (XCVT1), whereas plants under the XCVP3 treatment started releasing methyl salicylate on day 3 (Table 1). Furthermore, the amounts of methyl salicylate emitted by plants infected with the incompatible *Xanthomonas* were substantially higher than those emitted by plants infected with the compatible strain during the first 2 days of sampling (Table 1). A sharp burst in the release of this compound by plants under the XCVP3/BAW treatment on day 4 is noteworthy (Table 1).

Healthy (uninfected/undamaged; control) plants emitted relatively small VOC amounts throughout the sampling period (Fig. 1, Table 2). In contrast, plants infected with XCVT1, the incompatible strain, released amounts of volatiles that were higher than those emitted by XCVP3-infected plants and healthy control plants starting on the first day of collection

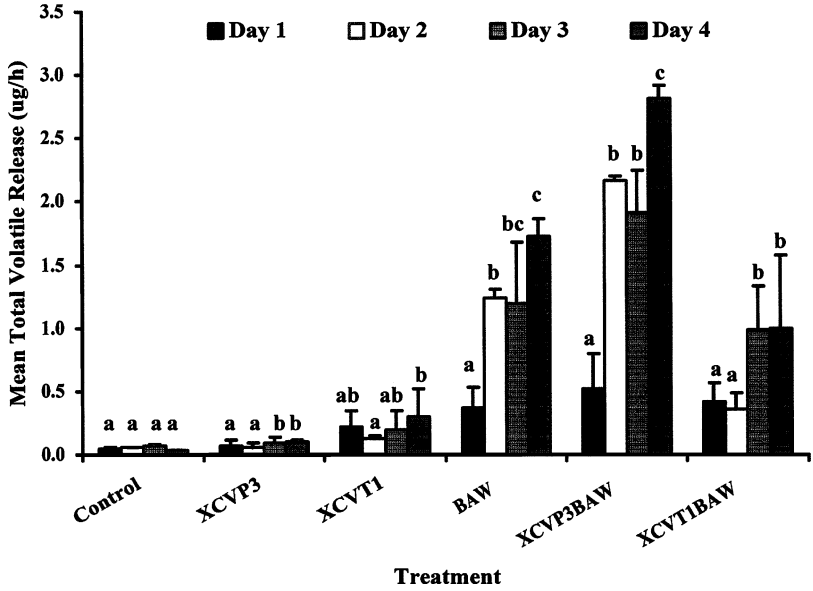

Fig. 1 Mean total volatile emissions from pepper plants for days 1–4 after bacterial inoculation. Bars within treatments headed by different letters denote significant differences between days (Tukey's mean separation test, $P = 0.05$). Error bars denote 1 SE

(Fig. 1). Emission of VOCs by plants exposed to the simultaneous bacterial and BAW challenge were higher than that of healthy plants or plants challenged by either bacterium alone (Fig. 1). Plants exposed to the XCVP3/BAW also released higher amounts of VOC than healthy plants exposed to BAW damage starting on the second day of collection, which contrasts sharply with amounts released by XCVT1/BAW-treated plants (Fig. 1).

Pepper plants exposed to the different bacteria/BAW treatments emitted distinct blends of compounds consisting of monoterpenes, homoterpenes, lypoxygenase products, linalool, methyl salicylate, and *cis*-jasmone. Pepper plants exposed to BAW feeding alone released a more complex VOC blend and a greater quantity of total volatiles, compared to healthy plants, or plants infected with either bacterium alone, starting on day 2 and persisting throughout the duration of the experiment (Table 1, 2). The VOC blend released by BAW damaged plants consisted of many compounds reported previously as induced by BAW feeding on other plant systems (Turlings et al., 1991; Turlings and Tumlinson, 1991; Röse et al., 1996; Cardoza et al., 2002) in addition to *Z*-3-hexenyl propionate, 2-methyl-hexyl-butyrate, 2-phenyl-ethyl-formate, *Z*-3-hexenyl-valerate, *Z*-3-hexenyl caproate, *Z*-3-hexenyl-hexenoate, β-elemene, α-selinene, and *Z*-3-hexenyl phenyl acetate (Table 2). Emission of VOC by plants infected with XCVP3, the compatible strain, was comparable to that of control plants through the third day after bacterial inoculation. However, on day 4 after inoculation, XCVP3-infected plants released higher amounts of β-ocimene, methyl salicylate, and α-selinene compared to healthy control plants (Table 2). The VOC profile of XCVT1-infected plants was for the most part composed of α-pinene, limonene, β-ocimene, *E*-2-hexenyl butyrate, β-elemene, and (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (Table 2). Plants under the simultaneous XCVP3/BAW challenge released a blend consisting mainly of *Z*-3-hexenyl acetate, β-myrcene, β-ocimene, *Z*-3-hexenyl propionate, methyl salicylate, and 2-

Table 2 Volatile emissions (mean ± SE) from pepper plants under different bacteria/BAW treatments

Compound	Mean total volatile release (ng plant^{-1} h^{-1})[a,b]					
	Control	BAW	XCVP3	XCVP3+BAW	XCVT1	XCVT1+BAW
Monoterpenes						
α-Pinene	**72 ± 45.8**	123 ± 48.8	**128 ± 89.8**	44 ± 13.3	**848 ± 426.9**	166 ± 71.1
β-Pinene	**17 ± 7.7**	38 ± 13.1	14 ± 4.6	17 ± 12.0	89 ± 3.5	23 ± 7.9
β-Myrcene	0 ± 0.0	**1542 ± 569.9**	0 ± 0.0	**737 ± 375.0**	0 ± 0.0	295 ± 136.6
Limonene	8 ± 3.8	30 ± 30.0	3 ± 1.5	40 ± 36.5	**129 ± 77.5**	22 ± 9.2
Eucalyptol	0 ± 0.0	101 ± 31.6	3 ± 2.3	127 ± 44.71	34 ± 13.8	47 ± 21.8
β-Ocimene	**22 ± 11.3**	**5358 ± 1660.1**	**158 ± 124.2**	**9210 ± 2629.7**	**155 ± 75.8**	**2766 ± 1082.1**
Linalool	14 ± 6.0	**622 ± 368.1**	13 ± 3.6	126 ± 100.0	43 ± 8.8	165 ± 92.9
Homoterpenes						
E-4,8 Dimethyl-1,3,7-nonatriene	0 ± 0.0	169 ± 40.8	1 ± 1.0	233 ± 65.0	41 ± 21.8	114 ± 32.3
(*E*,*E*)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene	**20 ± 7.9**	423 ± 130.0	**44 ± 15.3**	642 ± 191.2	**149 ± 52.1**	**2998 ± 1364.4**
Sesquiterpenes						
β-Caryophyllene	11 ± 4.0	60 ± 23.7	4 ± 2.3	53 ± 37.0	17 ± 8.1	21 ± 8.4
α-Humulene	**36 ± 12.2**	138 ± 44.8	**33 ± 13.8**	99 ± 37.6	34 ± 28.2	123 ± 45.2
β-Farnesene	6 ± 4.4	50 ± 15.3	7 ± 5.0	86.7 ± 25.3	20 ± 12.8	113 ± 41.5
Cadinene	0 ± 0.0	35 ± 10.1	1 ± 0.5	53 ± 16.0	12 ± 2.8	304 ± 252.8
β-Selinene	0 ± 0.0	13 ± 6.4	1 ± 1.1	140 ± 142.9	6 ± 2.22	380 ± 159.7
β-Elemene	0 ± 0.0	308 ±92.4	5 ± 2.7	493 ± 219.8	**254 ± 193.4**	**3183 ± 1505.6**
α-Selinene	13 ± 5.3	185 ± 172.5	**48 ± 8.0**	33 ± 14.8	31 ± 9.5	77 ± 23.3
α-Farnesene	0 ± 0.0	10 ± 4.2	0 ± 0.0	8 ± 7.0	0 ± 0.0	9 ± 4.1
Nerolidol	9 ± 3.3	92 ± 31.1	8 ± 2.64	123 ± 60.6	11 ± 2.9	110 ± 35.9

Lipoxygenase products						
E-2-Hexenal	0 ± 0.0	427 ± 184.6	5 ± 4.9	351 ± 115.94	11 ± 4.41	56 ± 28.4
Z-3-Hexen-1-ol	3 ± 1.9	134 ± 91.7	3 ± 3.2	68 ± 74.11	19 ± 7.5	97 ± 35.5
Z-3-Hexenyl acetate	4 ± 2.8	23 ± 4.8	2 ± 1.2	**1391 ± 1495.7**	59 ± 23.7	14 ± 6.1
E-2-Hexenyl acetate	1 ± 1.3	**647 ± 501.3**	3 ± 2.0	360.4 ± 219.6	71 ± 46.9	114 ± 88.0
Z-3-Hexenyl propionate	0 ± 0.0	**1199 ± 503.6**	0 ± 0.0	**1454 ± 515.17**	0 ± 0.0	260 ± 176.1
E-2-Hexenyl butyrate	2 ± 2.6	**1694 ± 644.9**	2 ± 1.9	159 ± 133.6	**117 ± 67.0**	377 ± 174.4
Z-3-Hexenyl butyrate	0 ± 0.0	551 ± 428.7	1 ± 0.63	82 ± 24.0	99 ± 63.8	21 ± 14.2
Z-3-Hexenyl isobutyrate	3 ± 2.1	43 ± 16.0	6 ± 3.2	64 ± 24.1	20 ± 1.6	17 ± 5.7
2-Methyl-hexyl-butyrate	11 ± 3.7	357 ± 137.6	5 ± 3.0	**679 ± 400.2**	8 ± 1.6	63 ± 40.5
2-Phenyl-ethyl-formate	4 ± 2.8	261 ± 154.7	2 ± 2.3	45 ± 20.5	8 ± 4.6	47 ± 37.4
Z-3-hexenyl valerate	1 ± 1.1	88 ± 35.9	1 ± 0.39	76 ± 42.9	3 ± 1.4	31 ± 27.7
E-2-Hexenyl valerate	0 ± 0.0	0 ± 0.0	3 ± 1.5	4 ± 3.1	4 ± 1.2	4 ± 4.1
Z-3-Hexenyl tiglate	**67 ± 73.0**	90 ± 62.3	0 ± 0.0	25 ± 10.0	0 ± 0.0	23 ± 16.1
Z-3-hexenyl caproate	0 ± 0.0	31 ± 30.8	0 ± 0.0	0 ± 0.0	0 ± 0.0	**930 ± 839.6**
Z-3-Hexenyl hexenoate	0 ± 0.0	153 ± 71.2	0 ± 0.0	311 ± 91.4	8 ± 3.5	**542 ± 350.3**
Z-3-Hexenyl-phenyl acetate	8 ± 8.8	202 ± 202.0	0 ± 0.0	13 ± 7.5	8 ± 9.2	148 ± 51.3
cis-Jasmone	5 ± 2.3	334 ± 56.3	2 ± 1.7	375 ± 141.7	14 ± 7.1	142 ± 48.4
Amino acid derivatives						
Methyl salicylate	0 ± 0.0	0 ± 0.0	**198 ± 22.4**	**2936 ± 1464.3**	62 ± 20.8	**472 ± 81.4**
Indole	7 ± 3.6	148 ± 21.1	3 ± 1.8	109 ± 53.7	0 ± 0.0	41 ± 25.7

Values for day 4 period 2 (12:00–6:00 P.M.).

[a] Mean ± SE for 6 replicates per treatment.

[b] Underlined boldface within columns indicate the six highest emissions within a treatment.

methyl-hexyl butyrate. The VOC profile of plants under the XCVT1/BAW treatment consisted mostly of β-ocimene, methyl salicylate, *Z*-3-hexenyl caproate, *Z*-3-hexenyl hexenoate, β-elemene, and (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (Table 2). The relatively large amounts of sesquiterpene compounds released by XCVT1/BAW-treated plants starting on the first day of collection is particularly interesting (Table 1, 2). It is also worth noting that, on the first day of collection, the amounts of sesquiterpenes released by plants under the XCVT1 infection alone were similarly higher than those of other treatments, except XCVT1/BAW.

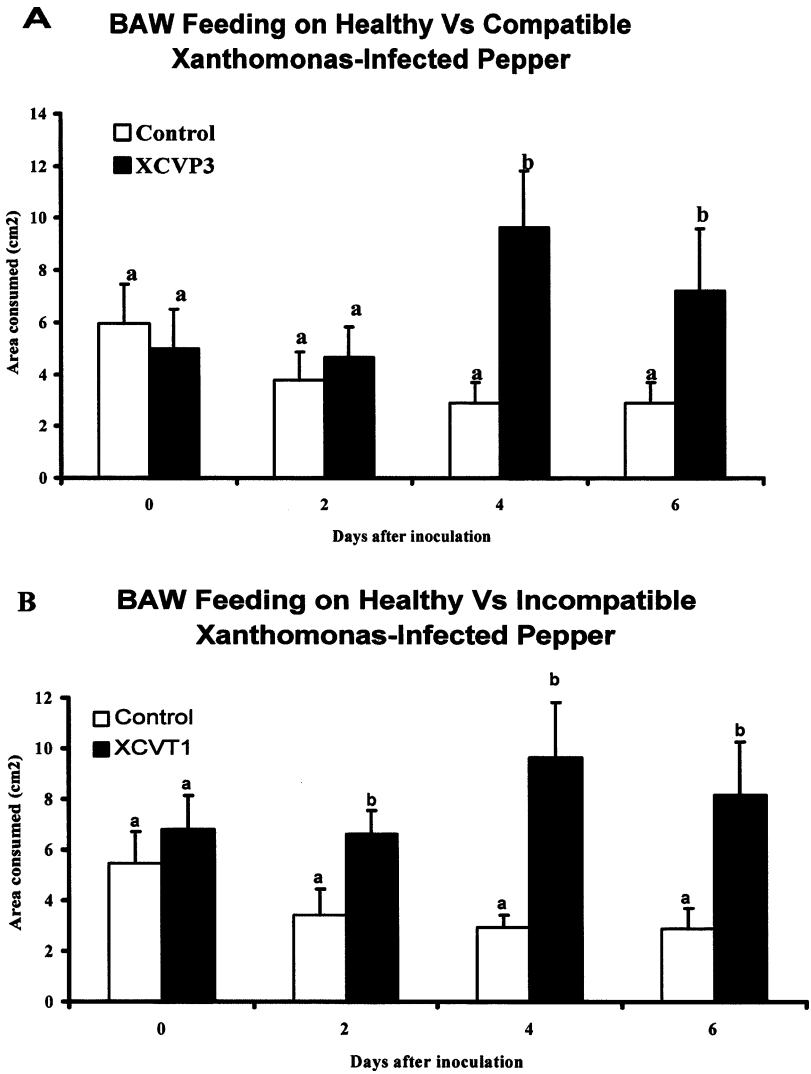

Fig. 2 Mean leaf area consumed by third instar *Spodoptera exigua* larvae in paired-choice tests with healthy (white bars) and *Xanthomonas* (thatched bars) plants at 0, 2, 4, and 6 d after bacterial inoculation. (a) Compatible (XCVP3) infection and (b) incompatible (XCVT1) infection. Error bars denote 1 SE and bars headed by different letters denote significant differences within days (paired *t* test, $P < 0.05$)

k include

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garozae

possibility is reinforced by the fact that the survival of the insects was higher when fed infected *versus* healthy pepper plants. Also, since insects reared on infected plants did not develop significantly faster, nor did they gain more weight, than those reared on healthy plants, the possibility of increased nutritional quality caused by the pathogen infection on the plant tissue is precluded. Our data are similar to previously reported findings, in that plant infection by a pathogen resulted in increased feeding and/or performance by an insect (Cardoza et al., 2002; Cui et al., 2002). The increase in insect feeding and performance on infected plants may have come as a consequence of biochemical changes induced by bacterial infection in the plants.

In recent years, there has been great interest in the potential applications of chemical elicitors to "vaccinate" plants to make them immune to attack or to "prime" them so their defense mechanisms are readily activated upon the first perception of pest damage. While chemical elicitation of plant defenses offers a promising alternative to environmentally harsh conventional pest control methods, caution needs to be exerted for there is a substantial body of evidence showing that the biochemical pathways involved in plant defense against pathogens and herbivores may interfere with one another. For example, recent studies have suggested that the plant pathway leading to jasmonate production and the salicylic acid pathway are involved in direct plant defense against pathogen invasion (Wasternack and Parthier, 1997; Thomma et al., 1998), and that the Jasmonic Acid (JA) and Salicylic Acid (SA) biochemical pathways may interfere with one another (Karban et al., 1987; Fidantsef et al., 1999; Stout et al., 1999; Bostock,1999; Felton et al., 1999). This theory has been substantiated by findings that some insect species prefer feeding on plants that are either infected by pathogens or induced with SA analog (Felton et al., 1999; Fidantsef et al., 1999; Stout et al., 1999; Thaler et al., 1999; Cui et al., 2002). Another fact supporting this theory is that acetyl salicylic acid and SA inhibit lipoxygenase-induced plant defenses by preventing JA accumulation (Peña-Cortes et al., 1993; Doares et al., 1995). Since we found that plant inoculation with either pathogen enhanced the development and survival of insects fed on infected plant tissue, it appears that the signaling cascade in response to pathogen infection in our system interfered with production of both direct and indirect defenses against the herbivore in this particular system. Additionally, reduction in plant emission of VOCs in response to BAW feeding seem to correlate with the timing of plant detection of each of the pathogen systems tested herein. Plant resistance to pathogens is governed by "gene-for-gene" interactions so that when a pathogen has an avirulence (*avr*) gene and a plant has the corresponding resistance (*R*) gene, the plant can immediately detect the pathogen and respond defensively (Hahn, 1996; Hammond-Kosack and Jones, 1997; Dangl and Jones, 2001). When the interaction ends in pathogen establishment and disease development, the interaction is considered compatible. If the pathogen fails to establish because of resistance from the plant, and no disease develops, the interaction is incompatible. Incompatible pathogen interactions induce defensive responses in plants at a much faster rate than compatible pathogen one. Thus, the reduced amount of BAW-induced VOC in XCVT1-infected plants may be attributable to plant resource allocation to fight off the pathogen.

Although studies addressing the issue of potential conflicts in plant defense responses to pathogens and herbivores now abound, to our knowledge, this is only the second report to evaluate VOC emissions in live plants in response to simultaneous pathogen/herbivore challenge. It is the first one to do so with different pathogen compatibility systems and at different times during the disease development process. We expect that studies such as this will enhance our understanding of the regulation of plant defenses in response to different aggressors and will lead to the development and implementation of methods for plant protection that use defense priming compounds. The hierarchy governing plant defense responses when under

attack by pathogenic and herbivorous organisms merits further attention. Similarly, the potential role of plant hormones and their interactions in biochemical cascades leading to plant volatile induction by multiple herbivorous organisms needs to be investigated.

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